

APPENDIX K4. Environmental Effects of Mosquito Control

ENVIRONMENTAL EFFECTS OF MOSQUITO CONTROL (USFWS, 2004)

This paper provides a summary of the potential impacts of mosquito control practices. This is not intended to discount the important role that mosquitoes play in the transmission of disease, nor is it intended to diminish the role that mosquito control can play in reducing the incidence of such vector-borne disease in humans. The first part discusses the ecological role of mosquitoes in the environment, aside from that as vectors of disease. The second part addresses the potential impacts of mosquito control pesticides to nontarget organisms and communities.

1. The Ecological Role of Mosquitoes

Mosquitoes are most often associated with their roles as vertebrate ectoparasites and vectors of disease-causing microorganisms. However, do mosquitoes provide any “beneficial” role in the environment, or are they, as Spielman and D’Antonio (2001) claim “self-serving” and of “no purpose other than to perpetuate her species”?

Mosquitoes As Prey

Larvae. Mosquitoes have evolved to use a wide variety of both permanent and temporary aquatic habitats for larval development. There are nearly as many habitats for mosquito larvae as there are types of lentic water bodies. For purposes of this discussion, mosquitoes will be divided into those that develop in ephemeral water bodies and those that develop in permanent to semi-permanent water.

The evolution of a drought- and sometimes freeze-resistant egg has allowed certain species of mosquitoes—the most common in the genera *Aedes*, *Ochlerotatus*, and *Psorophora*—to colonize a wide variety of ephemeral habitats large and small, from the tropics to sub-arctic zones. These mosquitoes lay eggs in dry or moist areas that will flood later. This strategy has at least two advantages: 1) the recently flooded detritus provides a nutrient-rich and abundant source of food for developing larvae; and 2) in many habitats there is a lag time before invertebrate predators colonize these temporary water bodies, allowing the larvae to develop in relatively predator-free environments. In most ephemeral habitats, mosquito eggs will hatch within hours of being flooded, often in very large numbers. In many of these habitats, such as summer flood pools and salt marshes, colonization by invertebrate predators occurs from highly mobile insects like dragonflies, beetles, and backswimmers that fly from more permanent bodies of water. Although some predators will arrive relatively quickly, it can take several days to weeks for an invertebrate predator community to become established. During the summer, a floodwater mosquito brood can develop from egg to adult in a week, and thereby mostly escape predation by these colonizing invertebrates.

In unpredictably flooded ephemeral habitats such as summer flood pools and storm-flooded salt marshes, there are few predators that have been identified to rely principally on mosquito larvae as a source of food. The unreliable nature of mosquito larvae as prey in these habitats prevents the development of any close predator-prey relationship unless the predator shares diapausing strategies similar to those of floodwater mosquitoes. The only predators in these habitats that rely on mosquito larvae for prey are other mosquitoes. A few species of *Psorophora* mosquitoes

have larvae that are predatory in late instar stages. These species are generally found in summer flood pools. Although there are few predators that specialize on mosquito larvae in these habitats, generalist predators such as beetles (larvae and adults), backswimmers, and some odonates (damselflies and dragonflies) will take advantage of the temporary abundance of mosquitoes if the timing of arrival into the habitats coincides with the presence of mosquito larvae.

Some ephemeral aquatic habitats, however, have flooding regimes that are more predictable. In at least two of these habitats, vernal pools and treeholes, we see the development of very close predator-prey relationships with mosquito larvae. In treeholes, species of another mosquito, *Toxorhynchites*, have evolved as predators of other treehole-dwelling mosquito larvae. Vernal pools in northern temperate regions predictably flood to their maximum extent in the early spring from rain and snowmelt, and this triggers a hatch of one or more species of *Ochlerotatus* mosquitoes. These are usually univoltine (single generation) species that laid eggs in the dry pool basin the previous summer. Hatching of mosquito larvae in vernal pools often occurs when water temperatures are still well below 10°C, with few predators active in such cold environments. The predators present at this time of the year are generally those that share similar overwintering strategies with mosquitoes, such as cyclopoid copepods (e.g., *Macrocyclops*) and a few species of beetles. Some species of predaceous diving beetles (family Dytiscidae) in the genus *Agabus* have evolved a diapausing strategy that closely resembles that of the *Ochlerotatus* mosquitoes. Unlike most dytiscid beetles, these species have drought- and freeze-resistant eggs that are laid in the dry basin the previous summer and hatch in the early spring concurrently with mosquitoes. The beetle larvae are active in the cold water and appear to feed primarily on mosquito larvae and pupae (Nilsson and Soederstroem 1988; Higgins and Merritt 1999). The predictable abundance of mosquitoes and general paucity of other potential prey species during the early spring in these pools has probably contributed to this specialization. Other predators in vernal pools will feed opportunistically on mosquito larvae. Some species of dragonflies and damselflies (Odonata, primarily *Sympetrum* and *Lestes*) have also evolved drought- and freeze-resistant eggs, but hatch later in the spring. Colonizing species of backswimmers (Notonectidae), water striders (Gerridae), and water beetles (Hydrophilidae and Dytiscidae) will feed on late-instar mosquito larvae and pupae, but are considered generalist predators (Higgins and Merritt 1999).

Mosquitoes that require water for oviposition include the common genera *Culex* and *Anopheles*. These mosquitoes colonize permanent to semi-permanent bodies of water, laying eggs on the surface. In many natural bodies of water, the larvae of these species must develop in the presence of an oftentimes-diverse invertebrate predator community. The co-occurrence of mosquito larvae and predatory invertebrates is more predictable in these habitats, but the diversity of other potential prey species may preclude the development of specialized predator-prey relationships. Potential invertebrate predators in these habitats include: backswimmers, water striders, giant water bugs (Belostomatidae), water measurers (Hydrometridae), adult and larval beetles (Dytiscidae, Hydrophilidae, Gyrinidae), many species of damselflies and dragonflies (Odonata), phantom midge larvae (Chaoboridae), and even copepods and flatworms. Although all of these predators can be considered generalists with regard to prey consumption, experimental evidence suggests that mosquito larvae, when available, are a preferred prey of some species (Helgen 1989; Urabe et al. 1990; Robert and Venkatesan 1997; Safurabi and Madani 1999).

Adults. Like other aquatic insects with terrestrial adult stages, mosquitoes provide a link between aquatic and terrestrial ecosystems as they convert detritus and aquatic microbial biomass into flying insect biomass. Most adult mosquitoes are relatively short lived. The probability of daily survival for adult mosquitoes, an important factor in disease transmission, varies among species and habitats. Daily survival probabilities usually range from 0.6-0.9, with much of the mortality coming from predation (Service 1993). Mosquitoes are fed upon by a variety of invertebrate predators, including spiders (Strickman et al. 1997; Fox 1998) and odonates (Sukhacheva 1996), although there are no known specialist predators that prey exclusively on mosquitoes. Vertebrate predators include insectivorous birds and bats (Zinn and Humphrey 1981), although mosquitoes often account for only a small percentage of the total biomass consumed. Consumption of mosquitoes by the Indiana bat, *Myotis sodalis*, for example, accounted for up to 6.6 percent of the total diet (Kurta and Whitaker 1998). The apparent absence of any specialized predator-prey relationships among adult mosquitoes and predators, however, does not necessarily discount the contribution of mosquitoes to the diet of a wide variety of generalized predators.

Other Ecological Roles of Mosquitoes

Mosquito larvae may feed by one or more of several different mechanisms. They may filter-feed, graze microbial biofilms, or even shred detritus (Merritt et al. 1992). In this sense, mosquitoes are a component of a functioning wetland ecosystem, processing detritus and aquatic microbes, and eventually providing a link between aquatic and terrestrial systems when they emerge.

Most adult mosquitoes require sugar meals during their lifetimes as a source of energy. The primary sources of sugars consumed by mosquitoes are nectar from flowers and honeydew excreted by aphids (Foster 1995). Both male and female mosquitoes frequently take nectar meals from flowers, but are they important as pollinators? Due to their small size and the limited probing abilities of the proboscis, mosquitoes are limited to feeding on nectar sources within flowers that have shallow or flat corollas. Unlike relatively large pollinators like bees and butterflies, mosquitoes can nectar feed efficiently without coming into contact with pollen-coated stamens. Thus, although they may transfer some pollen during the course of acquiring a meal of nectar, mosquitoes are probably not important pollinators in general (Foster 1995). A documented exception to this occurs in the subarctic where mosquitoes are significant pollinators of many plants (Kevan 1972).

The impact of reducing the density of mosquitoes in aquatic or terrestrial systems has not been studied. Generalist predators probably switch to alternate prey, which in turn may be impacted by the increased predation. The few specialist predators of mosquito larvae may be impacted the greatest due to the lack of alternate prey and/or the inability of such predators to uncouple from a closely evolved predator-prey relationship.

2. Nontarget Effects of Mosquito Control Pesticides

Mosquito control pesticides can be categorized into three groups: larvicides, adulticides, and water surface films (for controlling mosquito larvae and pupae). Compared with other forms of

pest control, there are relatively few pesticides available within each of these categories, and all differ with regard to efficacy and effects on nontarget organisms.

Larvicides

***Bacillus thuringiensis* var. *israelensis* (Bti).** Like other varieties of the natural soil bacterium, *Bacillus thuringiensis* (Bt), Bti is a stomach poison that must be ingested by the larval form of the insect in order to be effective. Bt contains crystalline structures containing protein endotoxins that are activated in the alkaline conditions of an insect's gut. These toxins attach to specific receptor sites on the gut wall and, when activated, destroy the lining of the gut and eventually kill the insect. The toxicity of Bt to an insect is directly related to the specificity of the toxin and the receptor sites. Without the proper receptor sites, the Bt will simply pass harmlessly through the insect's gut. Several varieties of Bt have been discovered and identified by the specificity of the endotoxins to certain insect orders. *Bacillus thuringiensis* var. *kurstaki*, for example, contains toxins that are specific to lepidopterans (butterflies and moths), while Bti is specific only to certain primitive dipterans (flies), particularly mosquitoes, black flies, and some chironomid midges. Bti is not known to be directly toxic to nondipteran insects.

Because Bti must be ingested to kill mosquitoes, it is much more effective on first-, second-, and early third-instar larvae than on late third and fourth instars since the earlier instars feed at a faster rate (fourth instar larvae feed very little). The pesticide is completely ineffective on pupae because they do not feed at all. Formulated products may be granular or liquid, and potency is expressed in International Toxicity Units (ITU), usually ranging from 200-1200 ITU. The concentrations of Bti in water necessary to kill mosquito larvae vary with environmental conditions, but are generally 0.05-0.10 ppm. Higher concentrations (0.1->0.5 ppm) of Bti are necessary when there is a high amount of organic material in the water, late-third and early fourth instar larvae predominate, larval mosquito density is high, or water temperature is low (Nayar et al. 1999). Operationally, Bti is applied within a range of volume or weight of formulated product per acre as recommended on the pesticide label, with the goal to achieve an effective concentration. The label recommended range of application rates under most conditions varies by a factor of 4 for most formulations (e.g., for granular formulations, 2.72-11.12 kg/ha (2.5-10 lb/acre)). For later instar larvae and water with a high organic content, higher application rates are recommended that may reach 8 times the lowest rate (e.g., for granular formulations, the higher rate is 11.1-22.5 kg/ha (10-20 lb/acre)). Mosquito control agencies use the recommended label rates, along with previous experience, to administer an effective dose. Typical application rates are often in the mid- to upper values of the normal ranges recommended on the labels (Abbott Laboratories 1999). Because water depths even within a single wetland can vary greatly, field concentrations of Bti can vary widely, especially when the pesticide is applied aerially. Efficacy is monitored by post-application reductions in mosquito larval density, but the actual concentration of Bti following an application is not measured. Thus, an insufficient concentration of Bti can be detected by low mortality of mosquito larvae, but an overdose (i.e., a concentration greater than necessary to kill mosquito larvae) of the pesticide is rarely monitored for.

The issue of Bti concentration is important with regard to impacts on nontarget organisms. Of particular concern is the potential for Bti to kill midge larvae (family Chironomidae). Chironomid (non-biting midge) larvae are often the most abundant aquatic insect in wetland

environments and form a significant portion of the food base for other wildlife (Batzer et al. 1993; Cooper and Anderson 1996; Cox et al. 1998). Negative impacts on chironomid density/biomass could have deleterious effects on wetland/wildlife food webs and could also lower biodiversity.

The potential for Bti to impact chironomid populations depends on the fate and availability of the pesticide, the ingestion of the pesticide, and the presence and number of specific receptor sites in the insect gut for the toxins (as discussed above). Fate and availability encompass both the initial dose/concentration and the fate of the pesticide in the aquatic environment. Chironomid larvae live primarily in the benthos of wetlands. Mosquito larvae ingest Bti primarily within the water column, but Bti readily adheres to suspended particulate matter and settles to the benthos (Yousten et al. 1992).

Ingestion of Bti by chironomid larvae depends primarily on the feeding mechanism. The family Chironomidae is a relatively large group, with nearly 1,000 species identified for North America (Merritt and Cummins 1996). This family encompasses a variety of feeding strategies: filter-feeders, collector-gatherers, scrapers, shredders, and even predators. Filter-feeding larvae are more likely to ingest Bti than larvae with other feeding strategies (Pont et al. 1999).

Chironomid larvae appear to possess mid-gut receptor sites for Bti endotoxins similar to those in mosquito larvae, and exhibit similar histopathological changes in the gut lining that lead to death of the insects when exposed to lethal concentrations of the pesticide (Yiallourous et al. 1999). There are, however, differences in the susceptibility of midge larvae to Bti at the subfamily level and among larval instars. In general, larvae in the subfamily Chironominae (Tribes Chironomini and Tanytarsini) are more susceptible to Bti than larvae of other subfamilies (Yiallourous et al. 1999) (Pont et al. 1999) (Ali 1981). Also, early-instar larvae are much more susceptible to Bti than later instars (Ali et al. 1981; Charbonneau et al. 1994).

There have been a number of laboratory and field studies examining the toxicity of Bti to chironomid larvae (Boisvert and Boisvert 2000). There have been many different formulations and potencies of Bti products used in these studies, and in many cases actual concentrations of Bti within the water were not measured. Also, differences in the species and instar of the chironomid larvae used (sometimes not specified), and in the environmental conditions of the field experiments make direct comparisons among the studies difficult. Most field studies examined the nontarget effects from a single application of Bti and did not address the potential long-term impacts from repeated applications over a season or over several seasons.

It is clear that in laboratory studies Bti is lethally toxic to some species of chironomid larvae at concentrations expected for mosquito control. Charbonneau et al. (1994) determined an EC_{50} (the concentration required to cause an effect in 50 percent of the test population) of 0.20 ppm for *Chironomus riparius* (fourth instar?), and the toxicity of Bti to earlier instars was over two orders of magnitude greater. Similarly, Ali et al. (1981) found the LC_{50} (the concentration required to kill 50 percent of the test population) for first-instar *Glyptotendipes paripes* (0.034 ppm) to be over two orders of magnitude lower than the LC_{50} for third instar larvae (8.31 ppm).

Charbonneau et al. (1994) studied the effects of Bti on chironomid larvae in the laboratory and the field. Laboratory toxicity tests on Chironominae larvae (the most susceptible subfamily)

demonstrated up to 100 percent mortality at label-recommended rates, but the toxicity of Bti to chironomids was influenced by several environmental factors. Factors that lowered toxicity to chironomids included higher water temperature, greater water depth, organic matter, and coverage by macrophytes. Field enclosure tests with Bti applied at 5.6 kg/ha (5 lb/acre) failed to demonstrate any pesticide effects on midge larvae within the enclosures, leading the authors to conclude that environmental factors reduce the toxicity of Bti to chironomids in the field. However, mortality of nontarget organisms within the enclosures was measured after 48 hours. Apparent effects of Bti on chironomids may not be detectable for 5-7 days post application (Ali 1981; Lacey and Mulla 1990; Pont et al. 1999). Also, because early instar larvae are much more susceptible to Bti, first and second instars would likely exhibit the greatest mortality. The 575 μ m mesh used to sample benthic invertebrates in the field tests of the Charbonneau et al. (1994) study, however, was too large to effectively sample first- and some second-instars. Thus, the conclusions regarding the field component of this study must be viewed with caution.

There is some evidence from field studies in which negative impacts to chironomid larvae were observed that such impacts are relatively short-lived (e.g., Miura et al. 1980). In most of these studies, however, it is not clear if the rebounding densities of midge larvae represent the same species or even the same subfamily that was initially reduced by the pesticide. Furthermore, population-level impacts to species from repeated applications over a season were usually not addressed. Although many species of chironomids are capable of producing several generations per year and could re-colonize a treated wetland relatively quickly, other species have only one generation per year and therefore would not be able to re-colonize until the following year. The ability of Bti-susceptible species to re-colonize a wetland following pesticide treatment would also depend on 1) the frequency of Bti applications, 2) the extent of Bti treatments within the wetland, and 3) the extent of Bti applications in the surrounding landscape. Widespread larviciding with Bti would provide few refugia for re-colonizing source populations of susceptible species.

In a study that examined population-level impacts to chironomids from a single application of Bti at a mosquito control rate, investigators showed that, while there was no statistical difference in the number of emerging adult chironomids between control and treatment enclosures, the species composition was different (Pont et al. 1999). Species sensitive to Bti (*Tanytarsus horni*, *T. fimbriatus*, and *Microchironomus deribae*) were 24-54 percent less abundant in enclosures treated at mosquito control rates than in control enclosures, while a less sensitive species (*Polypedilium nubifer*) was over 200 percent more abundant in the treated enclosure versus control. Higher application rates resulted in greater reductions of the Bti-sensitive species. This suggests that as Bti-sensitive chironomid larvae are killed by the pesticide, less sensitive species may thrive as they are released from competition (Pont et al. 1999). Thus, although chironomid larval numbers often appear to rebound after a treatment with Bti, this may be indicative of a shift in the species composition of the community, with species less sensitive to Bti replacing the sensitive species. It is unknown how or if such a shift would affect food web dynamics, but biodiversity would be lowered.

There is only one published study that examined the long-term, nontarget effects of Bti (Hershey et al. 1998; Niemi et al. 1999). In this study conducted in Minnesota, 27 wetlands were sampled for macroinvertebrates over a 6-year period. All wetlands were sampled for 3 pre-treatment years and randomly assigned to 3 treatment groups: Bti, methoprene (see discussion below), and

an untreated control group. The wetlands were sampled for 3 treatment years. Bti was applied to wetlands in a granular formulation at the rate of approximately 11.1 kg/ha (10 lb/ac), which represents the high end of the normal label-recommended application range. Bti was applied to each treatment wetland 6 times per year at intervals of 3 weeks or after rainfall of >1.25 cm, whichever came first (Niemi et al. 1999). Although this frequency of application is high, it is within the range that could occur from operational mosquito control.

After the first year of treatment, no differences in macroinvertebrate density, biomass, or community composition (richness of genera) among the treatments were observed (Hershey et al. 1998). However, in the second and third years of treatment, highly significant differences were observed in the two treatment groups compared to control. Chironomid larvae were significantly impacted by Bti treatments, with reductions in density of 66 percent and 84 percent for the second and third years of the study, respectively, compared to densities in control wetlands (Hershey et al. 1998). Significant declines in other nematoceran (primitive) dipteran larvae were also observed during the last two years of the study. There were also declines in macroinvertebrate predator densities in the Bti treated wetlands that the authors interpret as indirect effects from the reduction in a prey base dominated by chironomid larvae (Hershey et al. 1998; Niemi et al. 1999).

In summary, there is clear evidence from both laboratory and field studies that Bti can kill some chironomid larvae. Species in the subfamily Chironominae are apparently the most susceptible to direct toxicity; other subfamilies exhibit little mortality at mosquito control rates. Even within the subfamily Chironominae there are apparent differences among in susceptibility to Bti, relating perhaps to feeding mode (Pont et al. 1999). Within susceptible species, toxicity is greatest to early instars. Lethal concentrations of Bti are orders of magnitude lower for early versus late instars, and well within the concentrations expected from operational mosquito control. There is evidence that environmental conditions such as temperature, organic content of the water, vegetation, and density of larvae can ameliorate some of the potential negative impacts to chironomid larvae (Charbonneau et al. 1994), although field experiments designed to test this may be suspect.

The only long-term study on the nontarget effects of Bti for mosquito control demonstrated significant adverse effects on the chironomid community of treated wetlands, and this translated into numerous significant negative effects within the food web (Hershey et al. 1998; Niemi et al. 1999). The intensity of Bti applications used in this study, both the application rate and the frequency of applications, would represent the high end of those that would normally occur for operational mosquito control. In addition, entire wetlands were treated, which may or may not occur with aerial applications of Bti. Thus, the Minnesota study may represent a “worst-case scenario” of potential mosquito control operations, but it has generated the only data available on the long-term nontarget effects from Bti. Studies that examine nontarget effects of Bti from a single application or even within a single season may not be adequate to detect potential long-term impacts from pesticide use (Hershey et al. 1995).

There is also evidence that application rate can have a profound effect on impacts to chironomids from Bti (Rodcharoen et al. 1991). Because application rates of Bti for mosquito control can vary by a factor of 8, field concentrations of the pesticide can reach levels that are toxic to chironomid larvae, yet are still within the pesticide label directions. In addition, there are no

label restrictions on the number of applications of Bti to any one area. Economic considerations may preclude regular applications at the highest label rate, yet even at lower rates, adverse impacts to chironomid midge larvae have been demonstrated (Miura et al. 1980; Ali 1981; Ali et al. 1981).

Bacillus sphaericus

Bacillus sphaericus (Bsph) is a naturally-occurring soil bacterium similar to Bti, and has been developed as a commercially-available mosquito larvicide since the early 1990s. Like Bti, it releases a protein endotoxin in the alkaline gut of larval mosquitoes that attaches to specific receptor sites of susceptible species. This endotoxin dissolves the lining of the gut wall and eventually kills the larva. Unlike Bti, Bsph has only one endotoxin (Bti has two or more). Also, unlike Bti, Bsph is very effective in water with a high organic content, and is therefore often used in such habitats for control of *Culex* mosquitoes. Bsph is also capable of “cycling” in the aquatic environment, meaning it can retain its larvicidal properties after passing through the gut of a mosquito and—unlike Bti—provide effective mosquito control for weeks after a single application. Bsph, however, is not effective on all species of mosquitoes.

Because Bsph is a more recently developed larvicide than Bti, there are fewer studies that have examined the nontarget effects of this pesticide. The data available, however, indicate a high degree of specificity of Bsph for mosquitoes, with no demonstrated toxicity to chironomid larvae at any mosquito control application rate (Mulla et al. 1984; Ali and Nayar 1986; Lacey and Mulla 1990; Rodcharoen et al. 1991). This high specificity to some mosquito species and low toxicity to chironomid larvae is probably the consequence of the one endotoxin contained with the Bsph spore. Unfortunately, this also makes the development of resistance to this pesticide more likely if this pesticide becomes widely and frequently used.

Methoprene

Methoprene is a synthetic mimic of a naturally produced insect hormone, juvenile hormone (JH). All insects produce JH in the larval stages, with the highest levels occurring in the insect's early developmental stages. As an insect reaches its final stage of larval development, the level of JH is very low. This low level of JH triggers the development of adult characteristics. When an insect is exposed to methoprene, a hormonal imbalance in the development of the insect results, and it fails to properly mature into an adult. The insect eventually dies in the pupal stage. The most susceptible stages of development to methoprene are the later instars (for mosquitoes, third and fourth instars). In mosquito control applications, methoprene is applied directly to the larval breeding habitat. Larvae will continue to feed and may reach the pupal stage, but they will not emerge as adults. Methoprene is completely ineffective on mosquito pupae and adults. It is available in several formulations: liquid, granular, pellet, and briquet. There are several micro-encapsulated and extended-release formulations that remain effective for up to 150 days.

The amount of methoprene necessary for mosquito control is < 1.0 part per billion (ppb). The initial concentrations of methoprene when applied to aquatic habitats may reach 4-10 ppb, but residual concentrations are approximately 0.2 ppb (Ross et al. 1994). Once released into the aquatic environment, it is non-persistent, with a half-life of about 30-40 hours. Micro-encapsulated and extended-release formulations will, of course, be present in the water longer as

the pesticide is slowly released over time, 7-150 days, depending on the formulation. In field applications, efficacy is determined only by an observed inhibition of emergence of adults, since larvae are not directly killed by the pesticide.

Because methoprene is a JH mimic and all insects produce JH, there is concern about potential adverse impacts to nontarget aquatic insects when this pesticide is used for mosquito control. As with Bti, there is particular concern regarding potential negative impacts to chironomid larvae due to their importance in food webs. As with any pesticide, toxicity is a factor of dose plus exposure. At mosquito control application rates, methoprene is present in the water at very small concentrations (4-10 ppb, initially). With regard to exposure, chironomid larvae occur primarily in the benthos, either within the sediments and/or within cases constructed of silk and detritus. Thus, there may be differences with regard to exposure to methoprene between chironomid and mosquito larvae, the latter occurring primarily in the water column.

The published literature on the impacts of methoprene to chironomids is not as extensive as that for Bti. However, there is evidence for potential toxicity to chironomid and other aquatic invertebrates from methoprene treatments. Some early experiments indicated approximately 50 percent mortality of *Chironomus stigmaterus* (Chironomidae) and 70 percent of *Brachydeutera argentata* (Diptera: Ephidridae) larvae when exposed to 0.01 ppm of technical grade methoprene (Miura and Takahashi 1973). Mulla et al. (Mulla et al. 1974) noted up to 100 percent inhibition of emergence for some midge species, although the lowest concentration tested was 0.1 ppm. Breaud et al. (1977) observed reductions in several aquatic invertebrate taxa, including chironomids, after six applications of methoprene over an 18-month period in a Louisiana marsh. The application rate in this latter study was 0.028 kg/ha of active ingredient, although the formulation was not specified (Breaud et al. 1977).

In testing different formulations of methoprene against chironomids in experimental ponds, Ali (1991a) found that sustained-released formulations inhibited emergence of midges by 38-98 percent, in some cases for up to 7 weeks. A liquid, microencapsulated formulation applied at mosquito control rates resulted in a 60 percent inhibition of emergence in the tribe Chironomini for 14 days post-treatment. A pelletized, sustained-release (30 days) formulation applied at mosquito control rates inhibited all chironomid emergence by 64-98 percent for 7 weeks. A briquet formulation (30 days sustained-release) produced 38-98 percent inhibition of all chironomids for 7 weeks. The granular formulation applied at the high end of mosquito control rates reduced chironomid emergence by 61-87 percent (Ali 1991a).

In the multi-year Minnesota study cited above, a 3-week sustained-release, granular formulation of methoprene was applied to treatment wetlands at a label-recommended rate of 5-10 kg/ha (Hershey et al. 1998; Niemi et al. 1999). The pesticide was applied six times per season at 3-week intervals. The impacts from methoprene in this study were very similar to those observed for Bti. Negative impacts were not observed until the second and third years of treatment. In those years, significant declines in aquatic insect density and biomass were detected in methoprene-treated wetlands compared to controls. Total insect biomass was 70 percent and 81 percent lower in the second and third years of treatment, respectively, than in control wetlands (Hershey et al. 1998). Reductions were observed across many insect taxa, including predators and non-predators, suggesting direct (pesticide) and indirect (food web) effects from methoprene treatments (Hershey et al. 1998).

Although the application rate of methoprene used in the Minnesota study was well within operational rates used in mosquito control, the frequency of application exceeded what would probably occur under most field situations. Using a 3-week sustained release formulation and applying that every 3 weeks ensured a nearly constant exposure of methoprene to aquatic invertebrates in the treated wetlands throughout the season. Under such a scenario, it is unlikely that most impacted invertebrate populations would be able to re-colonize the wetlands during the treatments. However, this does not discount the conclusion that nontarget aquatic invertebrates were indeed impacted by methoprene at rates and concentrations used for mosquito control. Whether or not the observed food web effects would have been lessened under a more realistic pesticide application regime is debatable.

Studies of adverse impacts from methoprene on insect taxa other than chironomids are less conclusive. Because methoprene affects insect development and does not directly kill larvae, traditional toxicity testing over a few days is often inadequate when looking for potential impacts. Methoprene toxicity can only be observed at the point in which the immature insects reach (or fail to reach) adulthood. Thus, many published laboratory and field studies looking at nontarget impacts from methoprene were of insufficient duration to detect actual negative impacts (e.g., Miura and Takahashi 1973).

Breaud et al. (1977) observed adverse effects from methoprene on 14 aquatic invertebrate taxa, including *Callibaetis* sp. mayflies, odonates (dragonflies and damselflies), predaceous diving beetles, and chironomids. Negative impacts to *Callibaetis* mayflies from methoprene treatments have been observed by others (Steelman et al. 1975; Norland and Mulla 1975). Miura and Takahashi (1973) did not observe any mortality on *Callibaetis* from methoprene in laboratory or field studies, but neither was of sufficient duration (48 hours and 1 week, respectively) to adequately detect developmental effects (Miura and Takahashi 1973). Pinkney et al. (2000) observed consistently lower numbers of mayflies emerging from methoprene-treated wetlands compared to controls, but these differences were not statistically significant (Pinkney et al. 2000).

There is evidence of methoprene impacts to non-insects as well. McKenney and Celestial (1996) noted significant reductions in number of young produced in mysid shrimp at 2 ppb (McKenney and Celestial 1996). Sub-lethal effects on the cladoceran, *Daphnia magna*, in the form of reduced fecundity, increased time to first brood, and reduced molt frequency have also been observed at concentrations < 0.1 ppb (Olmstead and LeBlanc 2001).

There has been speculation and some preliminary data to suggest that methoprene causes limb malformations in amphibians (La Clair et al. 1998). However, experiments with methoprene and its degradation products have failed to demonstrate developmental toxicity even at concentrations exceeding 100 times that expected for mosquito control (Ankley et al. 1998; Degitz et al. 2003). Therefore, current data do not support a role of methoprene in amphibian malformations.

In summary, there is evidence for significant adverse nontarget effects from methoprene even when applied at mosquito control rates. With regard to negative impacts to chironomid midges, there may be differences in susceptibility among species and differences depending on the formulation used. One study in particular suggested that methoprene formulations with short-term residual activity may have smaller impacts to chironomids (Ali 1991a). However, even the

"ineffective" liquid formulation used in this study reduced emergence of Chironomini midges by 60 percent for two weeks. Certainly, not all midges will be affected by a single application of methoprene for mosquito control. However, the apparent differences in pesticide formulations, the varied susceptibility of species, and perhaps even the influence of some as-yet-undetermined environmental factors, make predicting the degree of any impacts nearly impossible.

Because methoprene does not immediately kill susceptible chironomid larvae, they are still available for predators. However, repeated applications of methoprene over a mosquito breeding season would eventually hinder recruitment as adults repeatedly fail to emerge (Hershey et al. 1998). Longer-term studies conducted over the course of a season or over multiple seasons are especially necessary for examining nontarget impacts from methoprene in order to detect potential impacts on longer-lived larvae (e.g., odonates, mayflies, and aquatic beetles) and to detect potential impacts to long-term recruitment. As was the case with Bti, the ability for a population to re-colonize a wetland following a methoprene treatment would depend on the intensity and frequency of applications at different spatial scales.

Temephos

Temephos is the only remaining organophosphate pesticide used for larval mosquito control. Like all organophosphate pesticides, it functions on the nervous system by inhibiting the production of acetylcholinesterase. Without this enzyme, nerves continue to fire, eventually resulting in death of the insect. Temephos is available in liquid or granular formulations that are applied directly to aquatic breeding habitats of mosquitoes. Expected environmental concentrations of temephos in water are 20-35 ppb, but actual field concentrations can vary widely (Pierce et al. 1996). Temephos is not persistent, but can remain effective for 7-10 days (Fortin et al. 1987).

There have been many studies examining the adverse nontarget impacts of temephos. Many of these studies have documented significant negative impacts to a wide range of aquatic taxa, especially in freshwater wetlands. Temephos is very highly toxic to cladocerans (water fleas, e.g., *Daphnia*) at fractions of expected mosquito control concentrations (Fortin et al. 1987; Helgen et al. 1988). The U.S. Environmental Protection Agency (EPA) has determined an LC₅₀ value of 0.01 ppb for *Daphnia magna* (EPA 1999), orders of magnitude lower than the expected environmental concentration of 20-35 ppb. The pesticide is highly toxic to chironomid larvae at or below mosquito control concentrations (Mulla and Khasawinah 1969; Iannacone and Alvarino 1998; Pinkney et al. 2000), although some researchers have documented only minimal effects on some species (Ali et al. 1978) (Ali 1991b). Temephos is especially toxic to larvae of the non-biting phantom midge, *Chaoborus* (Fales et al. 1968; Helgen et al. 1988; Pinkney et al. 2000). Temephos has also been found to be very toxic to potential mosquito predators such as odonates and backswimmers (Fales et al. 1968). Pinkney et al. (2000) reported significant reductions in insect diversity, richness, and density within temephos-treated experimental ponds, with significant declines in Ephemeroptera (mayflies), Odonata, Diptera, and Chironomidae compared to control ponds.

The effects of temephos on nontarget estuarine species are less studied. There are some data that suggest negative impacts from temephos are not as pronounced on estuarine species (Lawler et al. 1999b). However, there is evidence for toxicity to estuarine crustaceans from temephos at

concentrations below those expected from field applications for mosquito control (Mortimer and Chapman 1995; Brown et al. 1996). Studies have also shown sublethal and indirect impacts of temephos on fiddler crabs (Ward and Busch 1976; Pinkney et al. 1999). In addition, at least some chironomid species in salt marsh habitats are susceptible to temephos (Ali et al. 1992).

Temephos has also been shown to be lethal to tadpoles of green frogs at concentrations ≤ 10 ppb (Sparling et al. 1997).

It is clear that temephos is a much less specific larvicide compared to Bti and methoprene. Severe, negative impacts from temephos at mosquito control concentrations have been documented for a broad range of aquatic taxa in both freshwater and estuarine habitats, although some estuarine species are apparently more tolerant of the pesticide.

Surface Oils and Films

Surface oils and films are applied to mosquito breeding sites to kill mosquito larvae and pupae. The products create a barrier to the air-water interface and suffocate the insects, which require at least periodic contact with the water surface in order to obtain oxygen. The oils are mineral oil based and are effective for 3-5 days. Surface films are alcohol based and produce a monomolecular film over the water surface.

Both the oils and the films are potentially lethal to any aquatic insect that lives on the water surface or requires periodic contact with the air-water interface to obtain oxygen. Studies have demonstrated very significant negative impacts to water surface-dwelling insects from applications of oils (Mulla and Darwazeh 1981; Lawler et al. 1998).

Surface oils may also adversely impact wildlife by wetting the feathers of young waterfowl. This may be of particular concern at low temperatures when the oil could affect thermoregulation (Lawler et al. 1998).

Adulticides

All pesticides used to kill adult mosquitoes are broad-spectrum insecticides. The only selective aspect of these pesticides is in the manner in which they are applied. Most adulticides used currently are applied as ultra-low volume (ULV) sprays, meaning relatively small amounts are used (compared to some agricultural pesticides) and they are sprayed as very fine droplets (10-30 μ m in diameter). This small droplet size allows the spray to drift for a relatively longer period of time compared to larger droplets, and the small size delivers an appropriate dose of the pesticide to kill an adult mosquito. Drift is a necessary component of adulticiding because these sprays are most effective on flying insects. For this reason, adulticide applications generally occur in the evening or early morning hours when the majority of mosquito species are most active. Adulticides may be applied by truck-mounted sprayers or applied aurally by helicopter or fixed-wing aircraft.

There are only two general classes of adulticides: organophosphates and pyrethroids. Both classes of pesticides work on the nervous system, although have different modes of action. Organophosphates are cholinesterase inhibitors while pyrethroids are sodium channel blockers.

There are currently three organophosphate adulticides: malathion, naled, and fenthion, although fenthion is used only in a few counties in Florida and will be removed from the market in 2004. The most common pyrethroids are the synthetic pyrethroids, permethrin, resmethrin, and sumithrin. The pyrethroids are usually combined with the synergist piperonyl butoxide, which interferes with an insect's detoxifying mechanisms. None of these pesticides is persistent, with half-lives ranging from hours (naled) to several days (malathion and some pyrethroids).

Nontarget toxicity from adulticides may occur in either terrestrial or aquatic habitats as a result of deposition, runoff, inhalation, or ingestion. In general, pyrethroids have lower toxicity to terrestrial vertebrates than the organophosphates. With the exception of fenthion, which is highly toxic to birds, the application rates of the organophosphate adulticides are not likely to cause any direct mortality of vertebrates. Pyrethroids, although less toxic to birds and mammals, are very toxic to fish and aquatic invertebrates (Anderson 1989; Siegfried 1993; Milam et al. 2000). The actual toxicity of pyrethroids in aquatic habitats, however, is less than may be anticipated because of the propensity of these pesticides to adsorb to organic particles in the water (Hill et al. 1994). There are also data that indicate synthetic pyrethroid degradates have endocrine disrupting properties (Tyler et al. 2000).

In general, there are very few studies that have examined the nontarget effects of mosquito control adulticides. As all of these chemicals are broad-spectrum insecticides, they are potentially lethal to most insects. Yet there is a paucity of data available on the nontarget impacts to either terrestrial or aquatic invertebrates. There are data indicating the high toxicity of adulticides to honey bees (Taylor et al. 1987; Hagler et al. 1989; Pankiw and Jay 1992a; Pankiw and Jay 1992b), although the timing of adulticide applications in the evening can be expected to minimize these impacts.

Salvato (2001) examined the toxicity of naled, malathion, and non-synergized permethrin to 5 species of butterflies, including larval and adult stages. Naled and permethrin were found to be the most toxic to all life stages. The LD₅₀ data presented for some larvae and adults coincide with that delivered by a single ULV droplet of 5-23 μ m, within the desired range for mosquito control (Salvato 2001). Mosquito control adulticiding has been identified as a likely contributing factor in the decline of several rare lepidopteran species in the Florida Keys (Calhoun et al. 2000; Salvato 2001).

All adulticides are very highly toxic to aquatic invertebrates in concentrations < 1 ppb (Milam et al. 2000). Because most adulticides can be applied over or near water when used for mosquito control, there are risks to aquatic invertebrates from direct deposition and runoff of the pesticides. However, very few field studies have been conducted that have examined the impacts to aquatic organisms from mosquito control adulticides. Jensen et al. (1999) failed to detect reductions in aquatic invertebrate abundance or biomass from truck-mounted applications of pyrethrin, permethrin, and malathion. However, the potentially most sensitive group of invertebrates, cladocerans (water fleas), were not sampled (Jensen et al. 1999). This could be important given that malathion residues of 6 ppb were recovered from water in the treatment areas during this study. This is several times the LC₅₀ values of 0.69 ppb and 1.8 ppb of malathion for *Simocephalis serrulatus* and *Daphnia magna*, respectively (USEPA 2000), indicating that cladocerans would be at risk from applications of malathion for mosquito control.

Declines in flying insect abundance were also observed during this study following pesticide applications, but the numbers quickly rebounded (Jensen et al. 1999).

As was the case with studies of nontarget impacts from larvicides, the limited numbers of studies on adulticide impacts all involve examining short-term effects, usually from a single application of a pesticide. It is difficult to extrapolate the results of short-term experiments into predictions of long-term impacts, whether the short-term studies detected impacts or not. In addition, mosquito control is most often conducted at a landscape level. Studies of impacts at such larger temporal and spatial scales are non-existent, and would be a challenge both scientifically and economically.

Biological Control

The mosquitofish, *Gambusia affinis*, has been used for decades as a biological control of mosquito larvae. These fish are effective in removing mosquito larvae from relatively small, closed, and artificial aquatic systems, such as backyard ponds. In more complicated natural systems however, *Gambusia* are not selective predators, and can adversely impact native vertebrate and invertebrate communities (Rupp 1996). They can out-compete many native species of fish by feeding on eggs and fry, and they can actually reduce the density of natural invertebrate predators. There is also evidence that mosquitofish may cause direct and indirect impacts on tadpoles (Lawler et al. 1999a).

Summary/Conclusions

Mosquitoes are a natural component of many aquatic and terrestrial ecosystems. Like other aquatic insects with terrestrial adult stages, mosquitoes provide a link between aquatic and terrestrial habitats. Predation is probably the largest source of mortality for both larval and adult mosquitoes and, although there are relatively few predators that specialize on mosquitoes, these insects are fed upon by a wide variety of invertebrate and vertebrate predators. The impact of greatly reducing mosquito populations in aquatic and terrestrial ecosystems has not been studied.

Virtually every pesticide currently used to manage mosquito populations has the potential to adversely impact nontarget species. Widely used larvicides such as Bti and methoprene have been demonstrated to kill susceptible chironomid midge larvae, with experimental evidence suggesting that such population-level impacts may result in community-level food web effects. All adulticides are broad-spectrum insecticides that can potentially impact a wide variety of invertebrates and some vertebrates. The degree to which nontarget organisms or communities may be impacted by mosquito control pesticides is often difficult to predict because of differences in susceptibility among species, differences in toxicity of various formulated products, and basic knowledge gaps in toxicity data to certain species. An additional factor is the paucity of studies examining nontarget impacts of mosquito control at large spatial and temporal scales.

Organized mosquito control most often occurs at a landscape level such as a county or parish. When pesticides are applied to manage mosquito populations, it is often at multiple locations over relatively large spatial scales. Furthermore, pesticides may be applied to any given area multiple times in a season, year after year. The majority of nontarget mosquito control pesticide

studies have examined impacts at much smaller temporal and spatial scales, such as one application in a single wetland. While these studies provide useful data, it is difficult to extrapolate the results of these small-scale experiments into predictions of impacts from much larger scale treatments.

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